Tau Consortium 081418

Dennis Dickson—Mayo Clinic Brain Bank

* Distinct feature of PSP—tufted astrocytes
* CBD also has distinct astrocyte phenotypes
* These two might also have distinct tau degradation products/fragments
* CBD has more tau staining than PSP in forebrain, while PSP has more tau in hindbrain

Protein Structure Session—Bill DeGrado

* His mom had PSP ☹
* In collab with Prusiner lab
* Previous strain characterization: HDX, NMR, proteolysis, guanidine🡪all low throughput
* Fluorescent dyes are higher throughput reporter
* FLAMES—immobilize amyloids in agarose gel in wells
* Atsushi Ayogi from Prusiner lab has made an aB line analogous to Marc Diamond’s clone 1 line for use as aB detection/sensor line
* Infectivity seems to decrease with longevity; phosphorylation of three sites also seem to decay with age/longevity

Protein Structure Session—Marc Diamond

* Hypothesis: tau monomer transitions between two states, seed competent and incompetent
* Sonicated fibrils, used SEC to isolate fractions, found tau monomer fraction was seeding competent in the sensor line; isolated monomer from AD patient case
* In AD case, tau monomer that was isolated would form large assemblies spontaneously on the bench O/N
* At 4 weeks in P301L mice, soluble tau and “monomeric” tau have seeding activity
* Crosslinking MS to determine local and distant contacts in protein structure; unique crosslink in seed-competent monomer (150-230?) found both in recombinant tau and patient-derived tau
* Change in VQIINK sequences relative to each other in the XL-MS models
* AD samples seem to show only one strain by seeding clone strains, while CBD seems to form multiple distinct strains
* Apparently monomers can convert between strains?
* Big heat map—alanine scan to determine which aa’s are required for strain assembly (for ~20 strains)—unclear if these mutants are templating to preformed strains or are the starting material for each of these strains
* They were able to make the “seed-competent” monomer from recombinant protein (this was under the assumption that there are no PTMs in recombinant tau)
* According to collaborator Lukasz Joachimiak, monomeric “seed-competent” tau was prepared by incubating recombinant tau for 15 min with heparin (I believe RT/on the bench), followed by SEC🡪this leads to a 3mL shift in the elution profile of monomeric tau, compared to the control recombinant prep
* Unpublished: SEC-MALS has been used to verify that the elution of interest (seeding-competent tau) is monomeric

Protein Structure Session—Anthony Fitzpatrick

* Future directions: cryo-tomography, structural changes imparted by familial mutations, structures of oligomers, etc
* Could also try growing neurons on a grid, then imaging aggregates marked by fluorescent markers, then image the fibrils in the cells directly after thinning the sample (ex: Poly-GA aggregates, also sequesters proteasomes, see Cell 172(4):696-705, from this year)🡪this might be a method to determine mechanisms of toxicity

Protein Structure Session—Songi Han

* I like her, asked a good question to Marc Diamond about the possibility of his “monomer” being aggregated fragments
* Collaborates with Jenny Rauch in Ken Kosik’s lab
* Major question: what is the structural basis of seeding and emergence of pathways?
* Method: dipolar EPR to measure structural evolution (DEER)🡪this gives you distance distribution between spin labels (distinct structure yields narrow distribution of distance)
* Upon seeding with mouse fibril tissue (as opposed to heparin), the distribution got smaller/distances got shorter
* Heparin pathway: PNAS 2016 see that heparin addition transforms all/most monomer to oligomer almost immediately; distance distribution is very broad for heparin-induced fibrils—this means that the fibrils are very heterogeneous, instead of settling in one conformation
* See jenny’s work—mouse PHF can weakly seed tau in vitro agg, but second gen cannot seed tau in vitro
* Her lab has found it hard to seed in vitro with recombinant fibrils—apparently this requires sonication, but post-sonication tau is fragmented
* Hypothesis: tau fragment multimers are the “seeding-competent” unit
* Found addition of RNA cofactor allowed for seeding with fibrils in vitro (didn’t show any data with RNA alone)
* See tau-RNA droplet paper in PLoS Biol 2017, see also paper to come out with Jenny in the author list that defines a phase diagram for tau
* Marc Diamond: were able to separate fibrils into monomers by a “catalytic” method rather than by sonication—he found that sonication also caused fragmentation, so apparently they stopped doing that
* Bill DeGrado: R3 peptide shows some structure in slow exchange regime by NMR (so at least there may be structure in the repeat domains)—intrinsically disordered proteins can still for intra/intermolecular complexes🡪maybe this is the paper that Jason sent to read?

Protein Structure Session—Dan Southworth

* Hsp100 work in collab with Jim Shorter
* Eric’s work: CHIP-4R tau-Hsp70; stable and distinct complexes identified by SEC-MALS
* Hsp70-CHIP seems to form a stable tetramer
* Unclear about stoichiometry of the tetramer and tau—1 or 2 taus?
* Eric, Greg Merz, and Victor Banerjee—K18 P301L fibrils; still need to increase fibril separation to increase resolution🡪distance between beta sheets is ~4.5 angstroms
* P97 as a possibility for disaggregase before degradation by proteasome?

Protein Structure Session—Judith Steen

* MS/proteomics specialist, using patient samples and other models (IPSC, mouse models, etc)
* Their pipeline uses MASCOT and Skyline
* Used a method to visualize the data (similarity scores??), shows that some diseases show uniformity and others do not
* They use machine learning to identify each disease starting from brain tissue
* Can also identify peptides that are distinct in each disease
* Mapped 84 tau PTMs; AT8 epitope seemed to have low prevalence in patient data, some of the most prevalent PTMs don’t yet have Ab developed
* Hypothesis: tau cleavage accelerates or initiates aggregation
* Collab with Jenny in Kosik lab—fragments can become ThT pos and can seed aggregation in sensor cell lines
* Hypothesis about fragments: they have fewer degrees of freedom and lower entropic barrier to form beta sheet structure
* These core regions are also highly pos charged, neutralized by acetylation, heparin, RNA, etc
* Some proteases were found in tau aggregates—these are often expression induced by stress, and can cleave tau; these might arise due to traumatic injury (CTE, stroke, etc)
* Apparently they use DUBs to deacetylate their recombinant tau—need to check in about this (is it genetic, in vitro, does it remove all, need multiple DUBs?)

Protein Structure Session—Markus Zweckstetter

* Liquid phase separation of tau
* Tau phase separated in solution at 37C, not 5C
* Heparin addition changes the droplets—becomes more linked, instead of larger independent droplets; over 24 hours, converts to few very large droplets
* By measuring turbidity and CD (I think), K18, K19, and K25 show very distinct separation and structure
* See Ambadipudi Nat Comm 2017 for this data
* 1-2uM concentration and phosphorylation does lead to droplet formation—this concentration is relevant to tau concentration in neuron
* recently also observed tau binding to stress granules
* acetylated tau does not form the droplets, and no longer colocalizes with stress granules
* goal: look at the droplets by NMR
* it looks like CD or NMR was performed for tau at 5C vs 37C—seems that there are distinct changes in local structure
* hypothesis: tau phase separates and causes a super-saturation of tau in the droplets—it is the high concentration and potentially an inducer (RNA/interaction with RNA granules) that lead to aggregation of tau
* Marc Diamond: at nanomolar concentrations, he has had monomer that can convert to oligomers/higher order structures/seed
* 3R tau seems to have lower propensity to phase separate
* kinetics (Marc Diamond model) vs thermodynamic (Zweckstetter model)

Peter Walter

* ISRIB, screened for by Michelle for being able to effect integrated stress response
* Cognitive enhancer and anti-inflammatory
* ISRIB makes the GEF more active, and when it becomes limiting due to eIF2 phosphorylation, allows the GEF to still be active and translation of most mRNAs to continue
* ISRIB also reverses stress granule formation
* ISRIB binds eIF2B symmetrically in the middle of the octameric complex, and dimerization is how the drug does its activity
* At very high activation of integrated stress response, ISRIB no longer has activity (thus may be a reason for its low toxicity)🡪I think this is because most of the protein is now phosphorylated
* ISRIB increases spacial learning in mice (swimming assays, fear conditioning, etc)
* Integrated stress response must then limit memory consolidation, and ISRIB can release this brake
* Vanishing white matter disease: rare genetic disease linked to GEF mutation, has a neurodegenerative phenotype🡪ISRIB can rescue this function in vitro
* Karen Krukowski/Susanna Rosi—ISRIB improves learning in old mice

Ann McKee—CTE as a post-traumatic tauopathy

* Has distinct patterns of tau deposition in the brain from any other tauopathies, distinctly near blood vessels and near “crevices”
* Torsion due to high impact injuries affects the crevices the most—correlated with where tau gets deposited
* Starts out focally, but becomes widespread over time, independent of continued trauma
* Initial legions are hypophosphorylated (AT8-pos)
* CCL11—a biomarker, not sure what it hits

Daniel Perl—Tau pathology in military service members

* ~80% of TBI that happens during active service is not on the battlefield (ex: boxing is a required course at west point and naval academy)
* improvised explosive devices as weapons are an extensive source of TBI (responsible for ~60% of battlefield casualties)
* high pressure pulse blast wave can go through the skull and the brain
* phenotype: astroglial scarring
* most cases don’t show AT8 positivity in the brain, unless they are older (likely also had previous TBI)

Brain Trauma—Sam Gandy

* PET probe use in army veterans, couldn’t really follow this one
* BCI-838, an mGluR2/3 receptor antagonist, reverses phenotypes of TBI model mice🡪apparently by stimulating neurogenesis

Brain Trauma—Geoffrey Manley

* goal: track TBI patients over the long-term to watch the development of things like CTE

Brain Trauma—Susanna Rosi

* TBI rodent models: controlled cortical impact, close head injury, closed-head impact model
* Tests: working memory, spacial memory, social behavior, risk taking, executive function
* Focal contusion injury: hit the mice on the head in a controlled place, leads to increased integrated stress response
* Goal: is it possible to treat TBI after the time of injury, instead of before or at the same time
* Test molecule: ISRIB, only injected approx. 30 days post TBI; learning returned to levels of control, even a week after the treatment
* Mild concussion model: closed head injury (more diffuse, instead of localized to one spot in the brain)🡪still lead to activation of ISR
* At two weeks post diffuse injury, ISRIB treatment still was able to reverse learning deficit
* Repetitive mild head injury with full rotational acceleration of the head: 5 consecutive mild injuries every day for 5 days
* For this model, risk taking behavior persisted for up to 4 months (however, this was only seen in male mice, not female mice)
* ISRIB was treated ~1 month after the repetitive injuries—normal risk-taking behavior (1-3 months after treatment)

Brain Trauma—Gil Rabinovici

* From the MAC, tested tau PET tracer on football players
* FTC seems to be a very noisy tracer, high signal in normal controls, so shouldn’t be used to identify CTE